09-26-00

A

Practitioner's Docket No. 55225 ***PATENT***

Preliminary Classification:

Proposed Class:

Subclass:

NOTE: "All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P. § 601, 7th ed.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of

Inventor(s): Nicholas F. FRANANO

WARNING: *37 C.F.R. § 1.41(a)(1) points out:*

"(a) A patent is applied for in the name or names of the actual inventor or inventors.

"(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."

For (title): SYSTEMS AND METHODS FOR OPENING OBSTRUCTED BIOLOGICAL CONDUITS

CERTIFICATION UNDER 37 C.F.R. § 1.10*
(Express Mail label number is mandatory.)
(Express Mail certification is optional.)

I hereby certify that this New Application Transmittal and the documents referred to as attached therein are being deposited with the United States Postal Service on this date September 24, 2000, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EL440514716US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Peter Corless

(type or print name of person mailing paper)

Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

***WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

1. Type of Application

This new application is for a(n)

(check one applicable item below)

☒ Original (nonprovisional)

☐ Design

☐ Plant

WARNING: ***Do not*** *use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. § 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: ***Do not*** *use this transmittal for the filing of a provisional application.*

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

☐ Divisional.

☐ Continuation.

☐ Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. §§ 119(e), 120, or 121)

NOTE: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. § 112. Each prior application must also be:

(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or

(ii) Complete as set forth in § 1.51(b); or

(iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or

(iv) Entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f).

37 C.F.R. § 1.78(a)(1).

NOTE: If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. §§ 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. §§ 120, 121 or 365(c). (35 U.S.C. § 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. §§ 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

WARNING: *When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application* ***must*** *be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).*

☐ The new application being transmitted claims the benefit of prior U.S. application(s). Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required for filing date under 37 C.F.R. § 1.53(b) (Regular) or 37 C.F.R. § 1.153 (Design) Application

24 Pages of specification

5 Pages of claims

6

____ Sheets of drawing

WARNING: ***DO NOT*** *submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 C.F.R. § 1.84, see Notice of March 9, 1988 (1990 O.G. 57-62).*

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page . . ." 37 C.F.R. § 1.84(c)).

(complete the following, if applicable)

☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. § 1.84(b).

☐ formal

☐ informal

B. Other Papers Enclosed

____ Pages of declaration and power of attorney

____ Pages of abstract

____ Other

4. Additional papers enclosed

☐ Amendment to claims

☐ Cancel in this applications claims _____ before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

☐ Add the claims shown on the attached amendment. (Claims added have been numbered consecutively following the highest numbered original claims.)

☐ Preliminary Amendment

☐ Information Disclosure Statement (37 C.F.R. § 1.98)

☐ Form PTO–1449 (PTO/SB/08A and 08B)

☐ Citations

☐ Declaration of Biological Deposit

☐ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.

☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative

☐ Special Comments

☐ Other

5. Declaration or oath (including power of attorney)

NOTE: A newly executed declaration is not required in a continuation or divisional application provided that the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47, then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 C.F.R. §§ 1.63(d)(1)–(3).

NOTE: A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name including family name and at least one given name, without abbreviation together with any other given name or initial, and the residence, post office address and country or citizenship of each inventor, and state whether the inventor is a sole or joint inventor. 37 C.F.R. § 1.63(a)(1)–(4).

NOTE: "The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.62, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors." 37 C.F.R. § 1.41(a)(1).

☐ Enclosed

Executed by

(check **all** *applicable boxes)*

☐ inventor(s).

☐ legal representative of inventor(s). 37 C.F.R. §§ 1.42 or 1.43.

☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

☐ This is the petition required by 37 C.F.R. § 1.47 and the statement required by 37 C.F.R. § 1.47 is also attached. See item 13 below for fee.

☒ Not Enclosed.

NOTE: Where the filing is a completion in the U.S. of an International Application or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.

☐ Application is made by a person authorized under 37 C.F.R. § 1.41(c) on behalf of *all* the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 C.F.R. § 1.16(e) can be filed subsequently).

☐ Showing that the filing is authorized.
(not required unless called into question. 37 C.F.R. § 1.41(d))

6. Inventorship Statement

WARNING: *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

☐ The same.

or

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

☐ is submitted.

☐ will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of $130.00 required by 37 C.F.R. § 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 C.F.R. § 1.52(d).

☒ English

☐ Non-English

☐ The attached translation includes a statement that the translation is accurate. 37 C.F.R. § 1.52(d).

8. Assignment

☐ An assignment of the invention to _____

_____

☐ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

☐ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

WARNING: *A newly executed "CERTIFICATE UNDER 37 C.F.R. § 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.*

9. Certified Copy

Certified copy(ies) of application(s)

Country	Appln. No.	Filed
Country	Appln. No.	Filed
Country	Appln. No.	Filed

from which priority is claimed

☐ is (are) attached.

☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 C.F.R. § 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. § 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 C.F.R. § 1.16)

A. ☐ Regular application

CLAIMS AS FILED				
Number filed	Number Extra		Rate	Basic Fee 37 C.F.R. § 1.16(a) $690.00
Total Claims (37 C.F.R. § 1.16(c))	− 20 =	×	$ 18.00	
Independent Claims (37 C.F.R. § 1.16(b))	− 3 =	×	$ 78.00	
Multiple dependent claim(s), if any (37 C.F.R. § 1.16(d))		+	$260.00	

☐ Amendment cancelling extra claims is enclosed.

☐ Amendment deleting multiple-dependencies is enclosed.

☐ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 C.F.R. § 1.16(d).

Filing Fee Calculation $_____

B. ☐ Design application ($310.00—37 C.F.R. § 1.16(f))

Filing Fee Calculation $_____

C. ☐ Plant application
($480.00—37 C.F.R. § 1.16(g))

Filing fee calculation $_____

11. Small Entity Statement(s)

☐ Statement(s) that this is a filing by a small entity under 37 C.F.R. § 1.9 and 1.27 is (are) attached.

WARNING: *"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. § 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 C.F.R. § 1.28(a)(2).*

WARNING: *"Small entity status must not be established when the person or persons signing the . . . statement can* ***unequivocally*** *make the required self-certification." M.P.E.P., § 509.03, 6th ed., rev. 2, July 1996 (emphasis added).*

(complete the following, if applicable)

☐ Status as a small entity was claimed in prior application

_____ / _____, filed on _____, from which benefit is being claimed for this application under:

35 U.S.C. § ☐ 119(e),
☐ 120,
☐ 121,
☐ 365(c),

and which status as a small entity is still proper and desired.

☐ A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of **A, B** or **C** above)

$_____

NOTE: Any excess of the full fee paid will be refunded if small entitiy status is established and a refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under § 1.136. 37 C.F.R. § 1.28(a).

12. Request for International-Type Search (37 C.F.R. § 1.104(d))

(complete, if applicable)

☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

☒ Not Enclosed

☒ No filing fee is to be paid at this time.
(This and the surcharge required by 37 C.F.R. § 1.16(e) can be paid subsequently.)

☐ Enclosed

☐ Filing fee $ _____

☐ Recording assignment
($40.00; 37 C.F.R. § 1.21(h))
(See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION".) $ _____

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached
($130.00; 37 C.F.R. §§ 1.47 and 1.17(i)) $ _____

☐ For processing an application with a specification in
a non-English language
($130.00; 37 C.F.R. §§ 1.52(d) and 1.17(k)) $ _____

☐ Processing and retention fee
($130.00; 37 C.F.R. §§ 1.53(d) and 1.21(l)) $ _____

☐ Fee for international-type search report
($40.00; 37 C.F.R. § 1.21(e)) $ _____

NOTE: 37 C.F.R. § 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 C.F.R. § 1.53(f) and this, as well as the changes to 37 C.F.R. §§ 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f).

Total fees enclosed $_____

14. Method of Payment of Fees

☐ Check in the amount of $_____

☐ Charge Account No. _____ in the amount of $_____.
A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 C.F.R. § 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should not be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

☐ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. _____:

☐ 37 C.F.R. § 1.16(a), (f) or (g) (filing fees)

☐ 37 C.F.R. § 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: *Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.*

☐ 37 C.F.R. § 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

☐ 37 C.F.R. § 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a)).

☐ 37 C.F.R. § 1.17 (application processing fees)

NOTE: *". . .A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).*

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: *Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).*

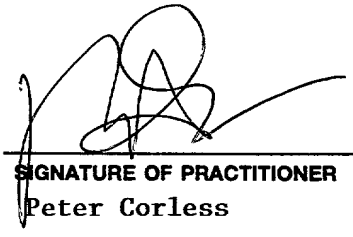
NOTE: *37 C.F.R. § 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . . the issue fee. . . " From the wording of 37 C.F.R. § 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.*

16. Instructions as to Overpayment

NOTE: ". . . Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☒ Credit Account No. 04-1105

☐ Refund



SIGNATURE OF PRACTITIONER

Reg. No. 33860

Peter Corless

(type or print name of attorney)

Tel. No. (617 523 3400

Edwards & Angell, LLP

P.O. Address

Customer No.

130 Water Street
Boston, MA 02109

☐ **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

☐ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added ______________

☐ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added ______________

☐ Plus added pages deleting names of inventor(s) named in prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added ______________

☐ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added ______________

☐ **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

☒ This transmittal ends with this page.

Docket No. 55225
Express Mail Label No. EL440514716US

SYSTEMS AND METHODS FOR OPENING OBSTRUCTED BIOLOGICAL CONDUITS

This application claims the benefit of U.S. Provisional Application Serial No. 60/155938 filed September 24, 1999, which is incorporated by reference herein in its entirety.

STATEMENT REGARDING GOVERNMENT RIGHTS

The U.S. Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention.

The present invention relates to methods of opening obstructed biological conduits. Preferred methods of the invention include methods and systems for opening obstructed biological conduits using local delivery of a therapeutic agent, particularly a protease, to lyse the extracellular matrix of the obstructing tissue.

2. Background.

Obstructions to biological conduits frequently result from trauma to the conduit which can result from transplant, graft or other surgical procedures wherein the extracellular matrix of the obstructing tissue largely comprises collagen. Balloon angioplasty is a common initial treatment for stenosis or stricture obstruction that yields excellent initial results (Pauletto, *Clinical Science*, (1994) 87:467-79). However, this dilation method does not remove the obstructing tissue.

It only stretches open the lumen, the trauma of which has been associated with the release of several potent cytokines and growth factors that can cause an injury which induces another round of cell proliferation, cell migration toward the lumen and synthesis of more extracellular matrix. Consequently, balloon angioplasty is associated with restenosis in nearly all patients (Pauletto, *Clinical Science*, (1994) 87:467-79). There is currently no treatment that can sustain patency over the long term.

The extracellular matrix, which holds a tissue together, is composed primarily of collagen, the major fibrous component of animal extracellular connective tissue (Krane, *J. Investigative Dermatology* (1982) 79:83s-86s; Shingleton, *Biochem. Cell Biol.*, (1996) 74:759-75). The collagen molecule has a base unit of three strands, of repeating amino acids coiled into a triple helix. These triple helix coils are then woven into a right-handed cable. As the collagen matures, cross-links form between the chains and the collagen becomes progressively more insoluble and resistant to lysis. When properly formed, collagen has a greater tensile strength than steel. Not surprisingly, when the body builds new tissue collagen provides the extracellular structural framework such that the deposition of hard collagen in the lesion can result in duct obstruction.

Benign biliary stricture results in obstruction of the flow of bile from the liver can result in jaundice and hepatic dysfunction. If untreated, biliary obstruction can result in hepatic failure and death. Billary strictures can form after duct injury during cholecystectomy. They can also form at biliary anastomoses after liver transplantation and other biliary reconstructive surgeries (Vitale, *Am. J. Surgery* (1996) 171:553-7; Lilliemoe, *Annals of Surgery* (1997) 225).

Historically, benign biliary stricture has been treated surgically by removing the diseased duct segment and reconnecting the duct end-to-end, or connecting the duct to the bowel via a hepaticojejunostomy loop (Lilliemoe, *Annals of Surgery* (1997) 225). These long and difficult surgeries have significant morbidity and mortality due to bleeding, infection, biliary leak, and recurrent biliary obstruction at the anastomosis. Post-operative recovery takes weeks to months. More recently, minimally invasive treatments such as percutaneous balloon dilation have been utilized, yielding good initial biliary patency surgeries (Vitale, *Am. J. Surgery* (1996) 171:553-7; Lilliemoe, *Annals of Surgery* (1997) 225). However, balloon dilation causes a localized injury, inducing a healing response that often results in restenosis (Pauletto, *Clinical Science*, (1994) 87:467-79). Long-term stenting at the common bile duct with flexible biliary drainage catheters is another minimally invasive alternative to surgery (Vitale, *Am. J. Surgery* (1996) 171:553-7). However, these indwelling biliary drainage catheters often become infected, or clogged with debris, and must be changed frequently. At present, long-term treatment of biliary stricture remains a difficult clinical problem.

Patients with chronic, end-stage renal failure may require replacement of their kidney function in order to survive. In the United States, long-term hemodialysis is the most common treatment method for end stage chronic renal failure in the U.S. In 1993, more- than 130,000 patients underwent long term hemodialysis (Gaylord, *J. Vascular and Interventional Radiology* (1993) 4:103-7), More than 80% of these patients implement hemodialysis through the use of a synthetic arteriovenous graft (Windus, *Am. J. Kidney Diseases* (1993) 21:457-71). In a majority of these patients, the graft consists of a 6 mm Gore-Tex tube that is surgically implanted between an artery and a vein, usually in the forearm or upper

arm. This high flow conduit can then be accessed with needles for hemodialysis sessions.

Nearly all hemodialysis grafts fail, usually within two years, and a new graft must be created surgically to maintain hemodialysis. These patients face repeated interruption of hemodialysis, and multiple hospitalizations for radiological and surgical procedures. Since each surgical graft revision consumes more available vein, eventually they are at risk for mortality from lack of sites for hemodialysis access. One estimate placed the cost of graft placement, hemodialysis, treatment of complications, placement of venous catheters, hospitalization costs, and time away from work at as much as $500 million, in 1990 alone (Windus, *Am. J. Kidney Diseases* (1993) 21:457-71).

The most frequent cause of hemodialysis graft failure is thrombosis, which is often due to development of a stenosis in the vein just downstream from the graft-vein anastomosis (Safa, *Radiology* (1996) 199:653-7. Histologic analysis of the stenosis reveals a firm, pale, relatively homogeneous lesion interposed between the intimal and medial layers of the vein which thickens the vessel wall and narrows the lumen (Swedberg, *Circulation* (1989) 80:1726-36). This lesion, which has been given the name intimal hyperplasia is composed of vascular smooth muscle cells surrounded by an extensive extracellular collagen matrix (Swedberg, *Circulation* (1989) 80:1726-36; Trerotola, *J. Vascular and Interventional Radiology* (1995) 6:387-96). Balloon angioplasty is the most common initial treatment for stenosis of hemodialysis grafts and yields excellent initial patency results (Safa, *Radiology* (1996) 199:653-7). However, this purely mechanical method of stretching open the stenosis causes an injury which induces another round of cell proliferation, cell migration toward the lumen and synthesis of more extracellular matrix. Consequently, balloon angioplasty is associated with restenosis in nearly all patients

(Safa, *Radiology* (1996) 199:653-7). There is currently no treatment which can sustain the patency of synthetic arteriovenous hemodialysis grafts over the long term.

Intimal hyperplasia research has focused largely on the cellular component of the lesion. The use of radiation and pharmaceutical agents to inhibit cell proliferation and migration are active areas of research (Hirai, *ACTA Radiologica* (1996) 37:229-33; Reimers, *J. Invasive Cardiology* (1998) 10:323-31; Choi, *J. Vascular Surgery* (1994) 19:125-34). To date, the results of these studies have been equivocal, and none of these new treatments has gained wide clinical acceptance. This matrix is composed predominantly of collagen and previous work in animals has demonstrated that systemic inhibition of collagen synthesis decreases the production of intimal hyperplasia (Choi, *Archives of Surgery* (1995) 130:257-261).

During normal tissue growth and remodeling, existing collagen matrices must be removed or modified. This collagen remodeling is carried out by macrophages and fibroblasts, two cell types which secrete a distinct class of proteases called "collagenases" (Swedberg, *Circulation* (1989) 80:1726-36; Trerotola, *J. Vascular and Interventional Radiology* (1995) 6:387-96; Hirai, *ACTA Radiologica* (1996) 37:229-33). These collagenases rapidly degrade insoluble collagen fibrils to small, soluble peptide fragments, which are carried away from the site by the flow of blood and lymph.

See also U.S. Patents 5,981,568; 5,409,926; and 6,074,659.

It thus would be desirable to provide new methods to relieve obstructions blocking flow through biolgical conduits.

SUMMARY OF THE INVENTION

I have now found new methods and systems for relieving an obstruction in a biological conduit, e.g. mammalian vasculature. Methods of the invention include administration to an obstruction site of a therapeutic agent that can preferably degrade (*in vivo*) the extracellular matrix of the obstructing tissue, particularly collagen and/or elastin. Preferred methods of the invention include administration to an obstruction of an enzyme or a mixture of enzymes that are capable of degrading key extracellular matrix components (including collagen and/or elastin) resulting in the solubilization or other removal of the obstructing tissue.

Methods and systems of the invention can be applied to a variety of specific therapies. For example, methods of the invention include treatment of bilary stricture with the use of exogenous collagenase, elastase or other agent, whereby an enzyme composition comprising collagenase, elastase or other agent is directly administered to or into (such as by catheter injection) the wall of the lesion or other obstruction. The enyzme(s) dissolves the collagen and/or elastin in the extracellular matrix, resulting in the solubilization of fibrous tissue from the duct wall near the lumen, and a return of duct flow or opening.

Methods of the invention also include pretreating an obstruction (e.g. in a mammalian duct) with collagenase, elastase or other agent to facilitate dilation such that if treatment under enzymatic degradation conditions alone is insufficient to reopen a conduit, then conventional treatment with e.g. balloon dilation is still an option. It has been found that enzymatic degradation pre-treatment in accordance with the invention can improve the outcome of balloon dilation since enzyme treatment partially digests the collagen fibrils. Therefore, the overall effect will be a softening of the remaining tissue. The softened tissue is more amenable to balloon

dilation at lower pressures, resulting in less mechanical trauma to the duct during dilation.

Preferably, the therapeutic agent is delivered proxumately to a targeted site, e.g. by injection, catheter delievery or the like.

A variety of therapeutic agents may be employed in the methods of the invention. Suitable therapeutic agents for use in the methods and systems of the invention can be readily identified, e.g. simply by testing a candidate agent to determine if it reduces an undesired vasculature obstruction in a mammal, particularly a coronary obstruction in a mammalian heart. Preferred therapeutic agents comprise one or more peptide bonds (i.e a peptidic agent), and typically contain at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acids, preferably one or more of the natural amino acids. Preferred therapeutic agents include large molecules, e.g. compounds having a molecular weight of at least about 1,000, 2,000, 5,000 or 10,000 kD, or even at least about 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000 or 100,000 kD.

Specifically preferred therapeutic agents for use in the methods and systems of the invention include proteases and other enzymes e.g. a collagenase e.g. Clostridial collagenase, a proteolytic enzyme that dissolves collagen, and/or an elastase such as a pancreatic elastase, a proteosytic enzyme that dissolves elastin. Preferred delivery of collagenase and other therapeutic agents of the invention include directly injecting the agent into the target lesion or other obstruction. Preferably, a homogeneous distribution of a therapeutic enzyme or enzyme mixture is administered to a target site with a drug delivery catheter. The therapeutic agent can then dissolve the key extracellular collagen components necessary to solubilize the obstructing tissue from the vessel wall near the lumen.

Treatment methods of the invention provide significant advantages over prior treatment methodologies. For example, enzymatic degradation of one or more key components of the extracellular matrix gently removes the tissue obstructing the lumen. Additionally, collagenolysis or other therapeutic administration is relatively atraumatic. Moreover, collagenase also can liberate intact, viable cells from tissue. Therefore, treatment methods of the invention can remove both the source of mechanical obstruction and a source of cytokines and growth factors, which stimulate restenosis.

A single or combination of more than one distinct therapeutic agents may be administered in a particular therapeutic application. In this regard, a particular treatment protocol can be optimized by selection of an optimal therapeutic agent, or optimal "cocktail" of multiple therapeutic agents. Such optimal agent(s) for a specific treatment method can be readily identified by routine procedures, e.g. testing selected therapeutic agents and combinations thereof in *in vivo* or *in vitro* assays.

In another aspect of the invention, treatment compositions and treatment kits are provided. More particularly, treatrment compositions of the invention preferably contain one or more enzmatic agents such as collagenase preferably admixed with a pharmaceutically acceptable carrier. Such compositions can be suitably packaged in conjuction with an appropriate delivery tool such as an injection syringe or a delivery catheter. The delivery device and/or treatment solution are preferably packaged in sterile condition. The delivery device and treatment composition can be packaged separately or in combination, more typcially in combination. The delivery device preferably is adapted for in situ, preferably localized delivery of the therapeutic agent directly into the targeted bioloigcal conduit obstruction.

Typical subjects for treatment in accordance with the invention include mammals, particularly primates, especially humans. Other subjects may be treated in accordance with the invention such as domesticated animals, e.g. pets such as dogs, cats and the like, and horses and livestock animals such as cattle, pigs, sheep and the like. Subjects that may be treating in accordance with the invention include those mammals suffering from or susceptible to biliary stricture including benign biliary stricture, stenosis of hemodialysis graft, intimal hyperlasia, and/or coronary obstruction, and the like. As discussed above, methods of the invention may be administered as a pre-treatment protocol before other therapeutic regime such as a balloon angioplasty; during the course of another therapeutic regime, e.g. where a therapeutic composition of the invention is administered during the course of an angioplasty or other procedure; or after another treatment regime, e.g. where a therapeutic composition of the invention is administered after an angioplasty or administration of other therapeutic agents.

Other aspects of the invention are disclosed *infra*.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 shows a common bile duct in a dog with a high grade stricture;

FIG. 2 shows a common bile duct in a dog with a high grade stricture after treatment;

FIG. 3 is a histology picture of a normal common bile duct from a dog;

FIG. 4 is a histology picture of a common bile duct stricture from a dog with a high grade stricture before treatment;

FIG. 5 is a histology picture of a common bile duct stricture from a dog after treatment with collagenase wherein the arrows denote the outer limit of collagen breakdown; and

FIG. 6 shows a normal common bile duct in a dog.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods of introducing a therapeutic agent that is capabile of degrading an extracellular matrix components to thereby facilitate the reopening of a constricted biological conduit. In particular, the invention provides for introduction to an obstructed bioliogical conduit of a therapeutic agent that degrades collagen and/or elastin. The present invention further provides methods of dialating a biological conduit by introducing a therapeutic agent into a biological conducit, preferably an isolated segment of the conduit.

In one embodiment of the present invention, the degradation of a stricture, lesion or other obstruction is accomplished by introducing one or more therapeutic agents that are capable of degrading one or more extracellular matrix components thereby facilitating the reopening of the constricted segment of the conduit. Major structural components of the extracellular matrix include collagen and elastin.

Preferred therapeutic agents for use in accordance with the invention are able to interact with and degrade either one or both of collagen and elastin.

As discussed above, a variety of compositions may be used in the methods and systems of the invention. Preferred therapeutic compositions comprise one or more agents that can solubilize or otherwise degrade collagen or elastin *in vivo*. Suitable therapeutic agents can be readily identified by simple testing, e.g. *in vitro*

testing of a candidate therapeutic compound relative to a control for the ability to solubilize or otherwise degrade collagen or elastin, e.g. at least 10% more than a control.

More particularly, a candidate therapeutic compound can be identified in the following *in vitro* assay that includes steps 1) and 2):

1) contacting comparable mammalian tissue samples with i) a candidate therapeutic agent and ii) a control (i.e. vehicle carrier without added candidate agent), suitably with a 0.1 mg of the candidate agent contacted to 0.5 ml of the tissue sample; and

2) detecting digestion of the tissue sample by the candidate agent relative to the control. Digestion can be suitably assessed e.g. by microscopic analysis. Tissue digestion is suitably carried out in a water bath at 37°C. Fresh pig tendon is suitably employed as a tissue sample. The tissue sample can be excised, trimmed, washed blotted dry and weighed, and individual tendon pieces suspended in 3.58 mg/ml HEPES buffer at neutral pH. See Example 1 which follows for a detailed discussion of this protocol. Such an *in vitro* protocol that contains steps 1) and 2) is referred to herein as a "standard *in vitro* tissue digestion assay" or other similar phrase.

Preferred therapeutic agents for use in accordance with the invention include those that exhibit digestion activity in such a standard *in vitro* tissue digestion assay at least about 10 percent greater relative to a control, more preferably at least about 20% greater digestion activity relative to a control; still more preferably at least about 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% greater digestion activity relative to a control in such a standard *in vitro* tissue digestion assay.

Appropriate therapeutic agents can comprise at least one and frequently several enzymes such that the therapeutic agent is capable of degrading both significant matrix components of tissue obstruction. Particularly preferable therapeutic agents will comprise either a collagenase or elastase or both. Specifically preferred are therapeutic agents comprising highly purified, injectable collagenase preparation suchs as produced from cultures of Clostridia histolyticum by BioSpecifics Technologies Corporation (Lynbrook, NY). This enzyme preparation is composed of two similar but distinct collagenases. The Clostridial collagenases cleave all forms of collagen at multiple sites along the helix, rapidly converting insoluble collagen fibrils to small, soluble peptides. Also preferable are therapeutic agents comprising elastase, particularly pancreatic elastase, an enzyme capable of degrading elastin. Trypsin inhibitors also can be suitably employed as the therapeutic agent in the methods of the invention.

In a further aspect of the present invention, the methods further include means to prevent damage to tissue that is not associated with conduit obstruction. Preferred enzymes incorporated in the therapeutic agents are large (> 100,000 kD) and diffuse slowly in the extracellular compartment after injection. Further, collagenases comprise a domain (in addition to the active site) which binds tightly to tissue. Consequently, these enzymes remain largely contained within collagen-rich target tissues after injection. Also, the enzyme's activity is quickly extinguished in the blood pool by circulating inhibitors. Therefore, injected collagenase, which diffuses from the interstitial compartment into the blood pool, will be rapidly inhibited, preventing systemic side effects.

Fragments of therapeutic agents also can be administered to a patient in accordance with the invention. For example, fragments of the above-mentioned collagenases and elastases can be administered to a patient provided such fragments

provide the desired therapeutic effect, i.e. degradation of obstruction of a biological conduit. As referred to herein, a collagenase, elastase or other enzyme includes therapeutically effective fragments of such enzymes.

In certain preferred aspects of the invention, the therapeutic agent(s) that are administered to a patient are other than a cytostatic agent; cytoskeletal inhibitor; an aminoquinazolinone, particularly a 6-aminoquinazolinone; a vascular smooth muscle protein such as antibodies, growth hormones or cytokines.

In specific embodiments, the degradation of elastin, an extracellular matrix component that contributes to tissue elasticity, is not desirable. Therapeutic agents comprising only enzymes, which do not degrade elastin, such as collagenases, can be employed. Therefore, the elastic properties of the conduit wall will likely be preserved after treatment.

In a preferred aspect of the invention, a therapeutic agent comprising at least one enzyme capable of degrading elastin, collagen or both is delivered to the targeted obstruction site with a catheter. Preferred catheters are capable of directly localizing a therapeutic agent directly into the extracellular matrix of the obstruction. Particularly preferable catheters are able of delivering accurate doses of therapeutic agent with an even distribution over the entire obstructed area of the conduit. One particularly preferred example of a catheter for use in the method of the present invention is the Infiltrator® catheter produced by InterVentional Technologies Corporation (IVT) (San Diego, CA), which delivers a precisely controlled dosage of a drug directly into a selected segment of vessel wall (Figure 1) (Reimers, *J. Invasive Cardiology* (1998) 10:323-331; Barath, *Catherterization and Cardiovascular Diagnosis* (1997) 41:333-41; Woessner, *Biochem. Cell Biol.* (1996) 74: 777-84). Using this preferred catheter a therapeutic agent can be delivered at

low pressure via a series of miniaturized injector ports mounted on the balloon surface. When the positioning balloon is inflated, the injector ports extend and enter the vessel wall over the 360° surface of a 15 mm segment of vessel. Each injector port is less than 0.0035 inch in size. Drug delivery can be performed in less than 10 seconds, with microliter precision and minimal immediate drug washout. The injected drug is delivered homogeneously in the wall of the vessel or duct (Figure 2). The triple lumen design provides independent channels for guidewire advancement, balloon inflation and drug delivery. Trauma associated with injector port penetration is minimal and the long-term histologic effects are negligible (Woessner, *Biochem. Cell Biol.* (1996) 74: 777-84). In addition, the device has been engineered such that the injector ports are recessed while maneuvering in the vessel. Additionally, the Infiltrator® catheter is capable of balloon inflation with sufficient force for angioplasty applications. The excellent control of drug delivery observed with Infiltrator® can be significant since preferred therapeutic agents of the present invention potentially can degrade collagen and/or elastin in nearly all forms of tissue in a non-specific manner.

In yet another embodiment of the present invention, a therapeutic dose is employed which will restore conduit flow while maintaining conduit wall integrity. Several parameters need to be defined to maximize method efficiency, including the amount of enzyme to be delivered, the volume of enzyme solution to be injected so that the reopening of the conduit occurs with a single dose protocol. Ideally repeat or multiple dosing is reserved only for patients who have an incomplete response to the initial injection.

In regards to the volume of therapeutic agent solution delivered, preferably the conduit wall is not saturated completely, as this can lead to transmural digestion and conduit rupture. Instead, the optimal dose is determined by targeting the

thickness of the wall (from the outside in) which needs to be removed in order to restore adequate flow, while leaving the remaining wall intact. An overly dilute solution will be ineffective at collagen lysis while an overly concentrated solution will have a higher diffusion gradient into the surrounding tissues, thereby increasing the risk of transmural digestion and rupture.

Collagenase doses are generally expressed as "units" of activity, instead of mass units. Individual lots of collagenase are evaluated for enzymatic activity using standardized assays and a specific activity (expressed in units/mg) of the lot is determined. BTC uses an assay that generates "ABC units" of activity. The specific activity of other collagenase preparations are sometimes expressed in the older "Mandel units". One ABC unit is roughly equivalent to two Mandel units.

Preferable doses and concentrations of enzyme solution are between 1000 and 20000 ABC units, more preferable are between 2500 and 10000 ABC units and enzyme doses of 5,000 ABC units in 0.5 ml of buffer are most preferred.

It will be appreciated that actual preferred dosage amounts of other therapeutic agents in a given therapy will vary according to e.g. the specific compound being utilized, the particular composition formulated, the mode of administration and characteristics of the subject, e.g. the species, sex, weight, general health and age of the subject. Optimal administration doses for a given protocol of administration can be readily ascertained by those skilled in the art using dosage determination tests, including those described above and in the examples which follow.

Therapeutic agents of the invention are suitably administered as a pharmaceutical composition with one or more suitable carriers. Therapeutic agents

of the invention are typically formulated in injectable form, e.g. with the therapeutic agent dissolved in a suitable fluid carrier. See the examples which follow for preferred compositions.

As discussed above, the methods and systems of the invention can be employed to treat (including prophylactic treatment) a variety of diseases and disorders. In particular, methods and systems of the invention can be employed to relieve or otherwise treat a variety of lesions and other obstructions found in common bile ducts or vascular systems. Methods of the invention are also useful to relieve lesions and other obstructions in other biological conduits including e.g. ureterer, pancreatic duct, bronchi, coronary and the like.

The invention also includes prophalytic-type treatment, e.g. methods to dialate a biological conduit whereby the increased conduit diameter obviates the potential of obstruction formation within a conduit. Temporary and partial degredation of the elastin component of a conduit wall reduces the elasticity of the conduit thereby facilitating modifications of the size and shape of the conduit. Introducing a dose of therapeutic agent in accordance with the invention into the lumen of an isolated conduit or some section thereof results in complete or partial diffusion of the therapeutic agent into the wall of the isolated conduit during a specified period of time. Subsequent pressurization of the treated region either while the region is still isolated or after removing the means of isolation increases the lumen diameter by dilation. Regeneration of the conduit elastin framework results in a conduit with a larger lumen diameter and without compromising the structural integrity.

Arteriovenous hemodialysis grafts are frequently placed in the arm of the patient such that blood can be withdrawn and purified blood returned through the

graft. Frequently the lumenal diameter of the venous outflow is smaller than the graft lumenal diameter. Development of a stenosis due to intimal hyperplasia can further reduce the lumenal diameter of the venous outflow such that an insufficient volume of blood passes through the venous outflow. To prevent intimal hyperplasia and stenosis formation, dilating the venous outflow vein using the above described method of partially degrading the elastin component of the vascular wall downstream of the site of graft implantation such that the lumenal diameter of the venous outflow is similar to or larger than the diameter of the interposed loop graft reduces the likelihood of forming of a stenosis due to intimal hyperplasia. Venous dialation can be preformed either before or after interposing a graft between the artery and vein.

All documents mentioned herein are incorporated herein by reference. The present invention is further illustrated by the following non-limiting examples.

Example 1: Tissue digestion analysis.

The protocol of the following example is a detailed description of a "standard in vitro tissue digestion assay" as referred to herein.

The rate of tissue digestion, which is composed mostly of collagen, by a mixture of collagenase and elastase, proteolytic enzymes with activity respectfully against collagen and elastin, was determined. Trypsin inhibitor was added to negate the affect of any residual trypsin activity. Briefly, fresh pig tendon was excised, trimmed, washed, blotted dry and weighed. Individual tendon pieces were suspended in 3.58 mg/ml HEPES buffer at neutral pH and various concentrations of enzymes were added. Iodinated radiographic contrast was added in various concentrations to some of the enzyme solutions. The tissue digestion was carried out in a water bath at 37°C. At various time points, the tendon pieces were removed from the enzyme solution, washed, blotted dry and weighed. Each time point was

derived from the average of three samples. The effect of enzyme concentration on tissue digestion rates was studied. As expected, increasing the concentration of enzymes in vitro increased the rate of tissue digestion (Figure 3). Buffer alone had no effect on the tissue. Extrapolating digestion rates in vitro to an in vivo situation has proven difficult. For Dupuytren's contractures, the effective dose for transecting fibrous cords in vitro was 500 ABC. However, the effective in vivo dose was 10,000 ABC units.

The effect of iodinated radiographic contrast material on tissue digestion rates was also studied (Figure 4). This study was performed to monitor enzyme delivery by mixing it with contrast prior to injection. These results demonstrate that Omnipaque 350 iodinated contrast material inhibits enzyme activity at radiographically visible (35%) concentrations, but not at lower (1-5%) concentrations (Figure 4). Similar results were observed with Hypaque 60 contrast.

Example 2. Determining dose dependant in vitro activity of a therapeutic agent including collagenase, elastase, and a trypsin inhibitor.

The effect of enzyme concentration on tissue digestion rates was studied (Figure 3). The "1x" tissue sample was treated with collagenase 156 Mandel units/ml + elastase 0.125 mg/ml + trypsin inhibitor 038 mg/mg, The "2x" sample was treated with collagenase 312 Mandel units/ml + elastase 0.25 mg/ml + trypsin inhibitor 0.76 mg/ml. The "5x" sample was treated with collagenase 780 Mandel units/ml + elastase 0.625 mg/ml + trypsin inhibitor 1.9 mg/ml. All digestion volumes were 0.5 ml. Increasing the concentration of enzymes in vitro increased the rate of tissue digestion (Figure 3). Buffer alone had no effect on the tissue. An effective in vivo dose was found to be 10,000 ABC units.

Example 3. Determining the effect of iodinated radiographic contrast material on tissue digestion rates facilitate monitoring enzyme delivery prior to injection of a therapeutic agent comprising a contrast material into a patient.

The "35% Omnipaque" tissue sample was treated with collagenase 156 Mandel units/ml + elastase 0.125 mg/ml + 0.38 trypsin inhibitor with 35% OmniPaque 350 contrast (volume:volume). The "5% Omnipaque" sample was treated with collagenase 312 Mandel units/ml + elastase 0.25 mg/ml + 0.76 trypsin inhibitor with 5% Omnipaque 350 (volume:volume). The "1% Omnipaque" sample was treated with collagenase 312 Mandel units/ml + elastase 0.25 mg/ml + 0.76 trypsin inhibitor with 1% Omnipaque 350. All digestion volumes were 0.5 ml. These results demonstrate that Omnipaque 350 iodinated contrast material inhibits enzyme activity at radiographically visible (35%) concentrations, but not at lower (1-5%) concentrations (Figure 4). Similar results were observed with Hypaque 60 contrast.

Example 4. Creating a stricture in the common bile duct of dogs and treatment of the resulting stricture with transcatheter intramural collagenase therapy.

Right subcostal laparotomy was performed in dogs to expose the gallbladder, which was then affixed to the anterior abdominal wall of 11 dogs (n=11). After 2 weeks, a single focal thermal injury was made in the common bile duct (CBD) using a catheter with an electrocoagulation tip placed through the gallbladder access. A 4.8 Fr biliary stent was placed to prevent complete duct occlusion in 7 animals. Stricture development was monitored with percutaneous cholangiography over five weeks. Collagenase was then directly infused into the wall of the strictured CBD using an Infiltrator drug delivery catheter (n=3). The Infiltrator has three arrays of microinjector needles mounted on a balloon which extend and enter the duct wall over the 360-degree surface. After treatment, internal plastic stents were placed in 2 animals. Explants of the CBD were

obtained the following day. H&E, trichrome, and elastin staining were used for histopathologic analysis.

CBD strictures were successfully created in 7/11 animals as determined by cholangiography (Figure 1). Failures were due to gallbladder leak (n=2) and perforation at the site of thermal injury (n=2). Histologic analysis of an untreated stricture demonstrated a thickened wall with a circumferential network of collagen bundles and associated lumenal narrowing (Figure 4). Strictures treated with collagenase demonstrated a circumferential lysis of collagen at the treatment site, with sparing of the normal duct, arteries and veins (Figures 2 and 5). All three animals developed bile leaks after treatment, two from the gallbladder access site and one from the treatment site. There was vascular congestion and inflammation in portions of the small bowel mucosa and peritoneum after treatment in all animals, to varying degrees.

Example 5: Relieve of strictures in the common bile duct of a patient.

A large dog was used as the patient such that under general anesthesia a cholecystostomy tract was created and the gallbladder was "tacked" to the abdominal wall with retention sutures. A cholangiogram was performed with Hypaque-60, using a marker catheter, in order to define the anatomy. Then, a flexible catheter with a bipolar electrode tip was constructed as previously described (Becker, *Radiology* (1988) 167:63-8). This catheter was inserted through the gallbladder (Figure 5) and positioned with its "hot" tip (arrow) in the distal common bile duct such that the catheter was pulled back and the treatment was repeated until a 1.0 cm length of duct was injured (Figure 6). Immediately after delivering the current there was a mild-moderate amount of smooth narrowing of the treated segment of duct (arrow), possibly due to spasm or edema. A pigtail nephrostomy drainage catheter was then inserted through the fresh cholecystotomy tract into the gallbladder. The distal end was closed with an IV cap and buried in the

subcutaneous tissue. The surgical wounds were then closed in a two-layer fashion.

After 7 days, a follow-up cholangiogram was performed to evaluate the thermally induced stenosis. A 20 gauge needle was used to percutaneous access then drainage catheter through the IV cap. A cholangiogram was performed demonstrating moderate-marked dilatation of the biliary tree (Figure 1). There was a high-grade stricture of the mid common bile duct, where the thermal injury had been made.

Strictures are created in five large dogs using the methods described above and in Example 4. In addition, an objective measurement of biliary patency (the Whitaker study) is made of the common bile duct, both before and after making a stricture. The Whitaker study is performed by injecting normal saline through a catheter positioned in the common bile duct. Flow rates are increased and pressure measurements are taken with until a peak pressure of 40 mmHg is reached.

The thermal lesions mature into fibrous strictures over a six week period. One animal is then sacrificed and a histologic assessment is made of the extrahepatic biliary tree. Samples are taken of the duct proximal to the lesion, the mid portion of the lesion (Figure 4), the lesion edge, and the duct distal to the lesion. Assessments of 1) duct morphology. 2) cell type and number, 3) the extent and appearance of the extracellular matrix, and 4) extent of epithelialization are made. A second animal is sacrificed after an additional 6 weeks after thermal injury and a similar analysis carried out.

A cholangiogram is performed to visually assess the stricture (Figure 1) and a Whitaker test is also performed on the remaining 3 dogs. Then, the Infiltrator catheter is then deployed within the lesion and 0.5 mL of collagenase preparation

(10,000 Units/ml) is injected into the wall of the lesion. On post-treatment day 1, a follow-up cholangiogram and Whitaker test are performed.

In cases where incomplete response is noted, a second treatment can be given and a second follow-up chlorangiogram and Whitaker test is performed the following day. Hepatic enzyme levels will be drawn to assess the effect of stricture and then treatment on hepatic function. Alternatively, incomplete response from collagenase can be followed up with subsequent angioplasty or a combined collagenase/angioplasty treatment.

After treatment with collagenase, a final cholangiogram is taken after 1 week (Figure 2). At this time, the animal is sacrificed and the extrahepatic biliary tree harvested. Histologic assessments are made of the bile duct proximal to the treated lesion, the mid portion of the treated lesion (Figure 5), the treated lesion edge, and the duct distal to the lesion. Assessments of 1) duct morphology, 2) cell type and number, 3) the extent and appearance of the extracellular matrix, and 4) extent of epithelialization were made. Figure 5 is a histology image of a common bile duct stricture after treatment. The arrows denote the outer limit of collagen breakdown. The histological examination of the treated common bile duct stricture demonstrates as circumferential lysis of collagen at the treatment site, while sparing damage to the normal duct, arteries and veins.

Example 6: Relieve of stenosis due to intimal hyperplasia of a synthetic hemodialysis graft.

Standard, untapered 5 mm diameter polytetrafluoroethylene (PFTE) loop grafts were interposed between the femoral artery and the femoral vein in the hind limbs of 25-35 kg dogs, as described previously (Trerotola, *J. Vascular and Interventional Radiology* (1995) 6:387-96). An end-to-end configuration had been

selected to facilitate optimal positioning of the catheter drug delivery balloon during treatment of a stenosis. Standard, cut-film angiography is performed one week after surgery to assess the arterial inflow, the artery-graft anastomosis, the vein-graft anastomosis, and the venous outflow. After this, routine physical examination of the grafts will be carried out to screen for patency. Twenty weeks after surgery, standard, cut-film angiography is performed to assess the lumenal diameter of the grafts and their venous outflow. At this time, a stenosis due to intimal hyperplasia is seen in the venous outflow with an associated pressure gradient (Trerotola, *J. Vascular and Interventional Radiology* (1995) 6:387-96). Then, using the first animal, the therapy delivery catheter is deployed within a graft and 5000 ABC units of collagenase in 0.5 ml is infiltrated into the wall of the lesion at the venous outflow. The catheter is flushed and the contralateral lesion receives 1 ml of saline, delivered in an identical manner. Nearly all collagenase activity is extinguished after 1-2 days such that the grafts are re-examined with angiography after 3 days. Repeat measurements of lumenal diameter and invasive pressure measurements across the lesion are also taken. The animals are sacrificed and the grafts excised, pressure-fixed, and examined histologically. Assessments are made of the distal graft, the venous anastomosis, the mid-portion of the treated lesion, the lesion edge, and the normal vein downstream from the graft. Additional assessments of 1) cell type, morphology and number, 2) extent of extracellular matrix, 3) overall adventitial, medial, and intimal thickness, 4) extent of intimal hyperplasia, and 5) extent of endothelialization are made.

Example 7:

Four dogs are used for a controlled study of collagenase treatment. Bilateral grafts are created as described previously and standard, cut-film angiogaphy is performed one week after surgery to access the arterial inflow, the artery-graft anastomosis, the vein-graft anastomosis, and the venous outflow. After this, routine

physical examination of the grafts are carried out to screen for patency. Then, twenty weeks after surgery, standard, cut-film angiogaphy is performed to assess the lumenal diameter of the grafts and their venous outflow. An obvious stenosis due to intimal hyperplasia is usually seen in the venous outflow with an associated pressure gradient (Trerotola, *J. Vascular and Interventional Radiology* (1995) 6:387-96). The Infiltrator catheter is then deployed within the lesion and the selected dose of collagenase is infiltrated into the wall of the lesion. The contralateral, control graft is treated in an identical manner, except saline will be delivered instead of collagenase. Three days after treatment, the grafts are restudied with an angiography and invasive pressure measurements to determine the acute effects of collagenase treatment. Changes in lumenal diameter and pressure gradients are calculated for both the collagenase-treated group and the saline-treated group and ten days after collagenase treatment, the grafts are studied a final time. The animals will be sacrificed and the grafts will be excised, pressure-fixed, and examined histologically, as described above.

The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of this disclosure, may make modifications and improvements within the spirit and scope of the invention as set forth in the following claims.

What is claimed is:

1. A method for treating a obstructed biological conduit, comprising administering to the conduit an agent that can degrade extracellular matrix of obstructing tissue.

2. The method of claim 1 wherein the agent can solubilize or othwerwise degrade collogen or elastin.

3. The method of claim 1 wherein the agent can solubilize or otherwise degrade collogen.

4. The method of claim 1 wherein the agent can solubilize or otherwise degrade elastin.

5. The method of any one of claims 1 through 4 wherein the agent comprises an enzyme or a mixture of enzymes that can degrade collagen and/or elastin.

6. The method of any one of claims 1 through 5 wherein in a standard *in vitro* tissue digestion assay the agent exhibits at least about 10 percent greater digestion activity relative to a control.

7. The method of any one of claims 1 through 5 wherein in a standard *in vitro* tissue digestion assay the agent exhibits at least about 50 percent greater digestion activity relative to a control.

8. The method of any one of claims 1 through 7 wherein the agent is a collagenase, elastase or trypsin inhibitor.

9. The method of any one of claims 1 through 8 wherein the agent is administered by a catheter.

10. The method of any one of claims 1 through 9 wherein the obstruction of the biological conducit is a stenosis, stricture or lesion.

11. The method of any one of claims 1 through 10 wherein the biological conduit is an artery, vein, ureteter, bronchi, bile duct, or pancreatic duct.

12. The method of any one of claims 1 through 11 wherein the agent is administered to a mammal having an obstructed biological conducit, or susceptible to an obstructed biological conduit.

13. A method of dialating a biological conduit, comprising:

administering to a biological conducit a therapeutic agent that is capable of degrading elastin and/or collogen.

14. The method of claim 13 further comprising, after administering the therapeutic agent, pressurizing the biological conduit.

15. The method of claim 14 wherein the biological conduit is pressurized by mechanical action.

16. The method of claim 14 or 15 wherein the biological conduit is pressurized by a balloon catheter.

17. The method of any one of claims 14 through 16 wherein the therapeutic agent is administered and the pressurizing is performed by the same device.

18. The method of any one of claims 14 through 17 wherein, after administering the therapeutic agent, a time period is permitted to lapse sufficient for the administered therapeutic agent to permeate through walls of the biological conduit.

19. A pharmaceutical kit comprising:

an agent that can degrade extracellular matrix of obstructing tissue of a mammalian biological conduit;

a delivery device for the agent.

20. The kit of claim 19 wherein the agent can solubilize collogen and/or elastin.

21. The kit of claim 19 wherein the agent comprises an enzyme or a mixture of enzymes that can degrade collagen and/or elastin.

22. The kit of any one of claims 19 through 21 wherein the therapeutic agent is a collagenase, elastase or trypsin inhibitor.

23. The kit of any one of claims 19 through 22 wherein in a standard *in vitro* tissue digestion assay the agent exhibits at least about 10 percent greater digestion activity relative to a control.

24. The kit of any one of claims 19 through 23 wherein the device is a syringe or catheter.

25. A method for treating a mammal suffering from or susceptible to a disease or disorder associated with obstruction of a biological conduit of the mammal, comprising administering to the mammal a composition agent that can degrade the conduit obstruction.

26. The method of claim 25 wherein the composition degrades extracellular matrix of tissue of the conduit obstruction.

27. The method of claim 25 or 26 wherein the composition can solubilize or otherwise degrade collogen or elastin.

28. The method of any one of claims 25 through 27 wherein the composition comprises an enzyme or a mixture of enzymes that can degrade collagen and/or elastin.

29. The method of any one of claims 25 through 28 wherein the composition comprises collagenase, elastase and/or trypsin inhibitor.

30. The method of any one of claims 25 through 29 wherein in a standard *in vitro* tissue digestion assay the agent exhibits at least about 10 percent greater digestion activity relative to a control.

31. The method of any one of claims 25 through 30 wherein the therapeutic agent is administered by a catheter.

32. The method of any one of claims 25 through 31 wherein the obstruction of the biological conducit is a stenosis, stricture or lesion.

33. The method of any one of claims 25 through 32 wherein the biological conduit is an artery, vein, ureteter, bronchi, bile duct, or pancreatic duct.

34. The method of any one of claims 25 through 33 wherein the mammal is suffering from benign biliary stricture, stenosis of hemodialysis graft, intimal hyperlasia, or coronary obstruction.

35. The method of any one of claims 25 through 34 wherein the mammal is a human.

36. A method for treating a mammal suffering from or susceptible to biliary stricture, stenosis of hemodialysis graft, intimal hyperlasia, or coronary obstruction, comprising administering to the mammal a composition agent that can solubilize or otherwise degrade collogen or elastin of the mammal.

37. The method of claim 36 wherein the composition comprises an enzyme or a mixture of enzymes that can degrade collagen and/or elastin.

38. The method of claim 36 or 37 wherein the composition comprises a collagenase, elastase or a trypsin inhibitor.

39. The method of any one of claims 36 through 38 wherein the mammal is a human.

ABSTRACT

The invention provides methods to treating a obstructed biological conduit, that include administering to the conduit an agent that can degrade extracellular matrix of obstructing tissue. Particular methods include delivery of an enzyme or a mixture of several enzymes to the area or region of obstruction wherein the enzyme(s) have the capability to degrade extracellular matrix components within the obstruction thereby restoring the normal flow of transported fluid through the conduit. The invention also includes preventively dialating a section of conduit to minimize the risk of obstruction formation.

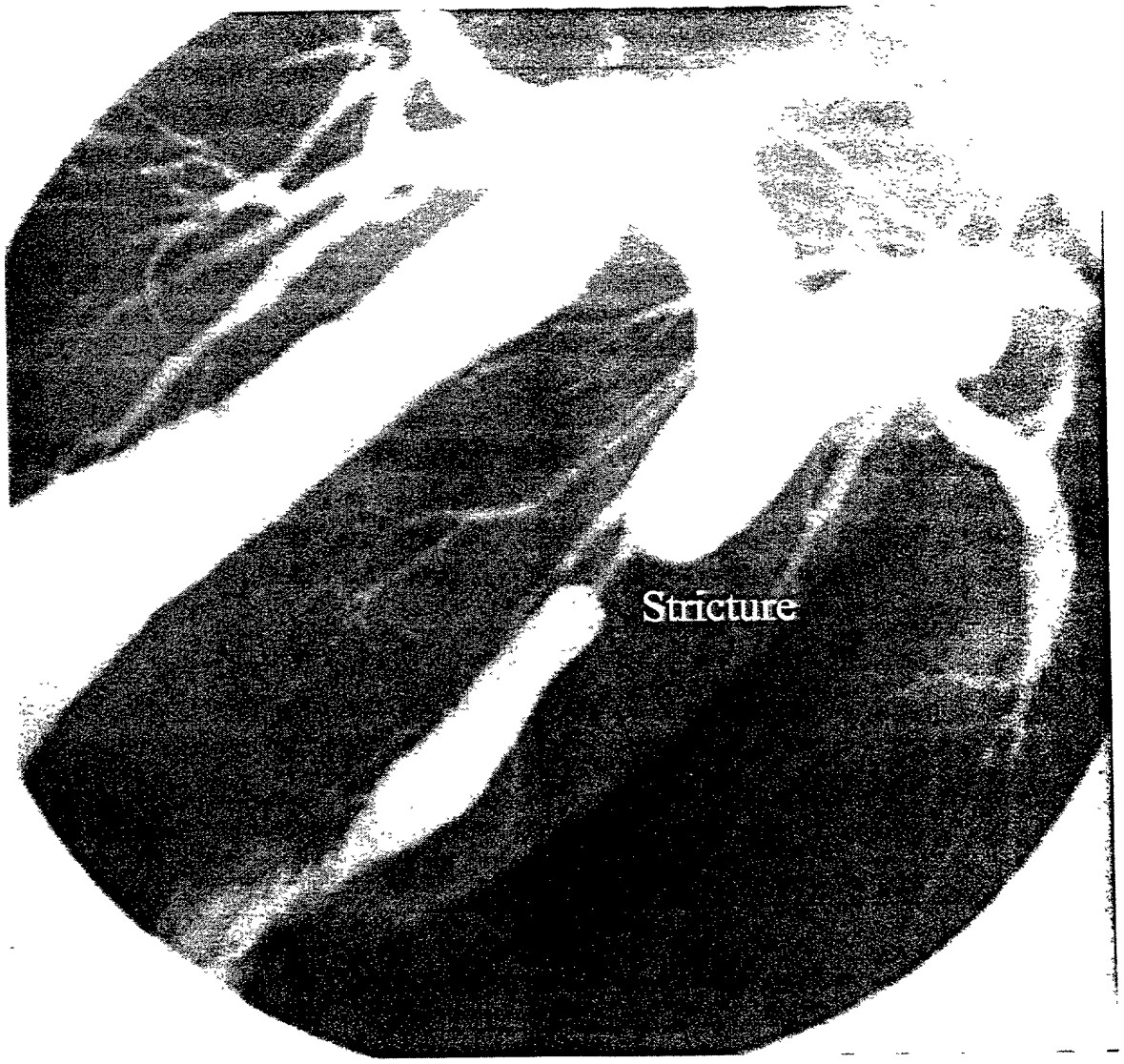


FIG. 1

FIG. 2

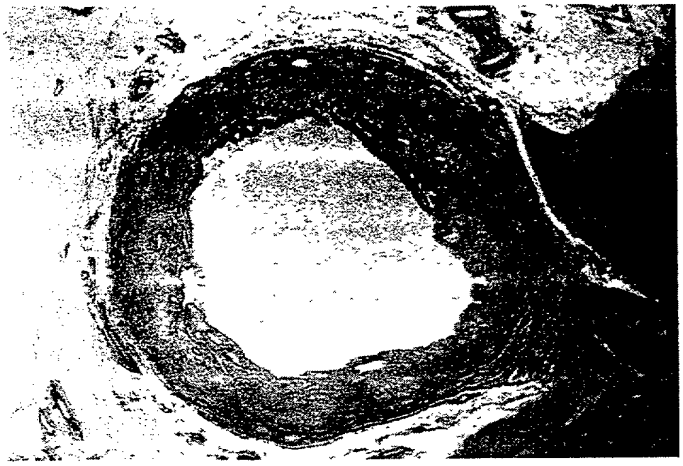


FIG. 3

FIG. 4

Stricture

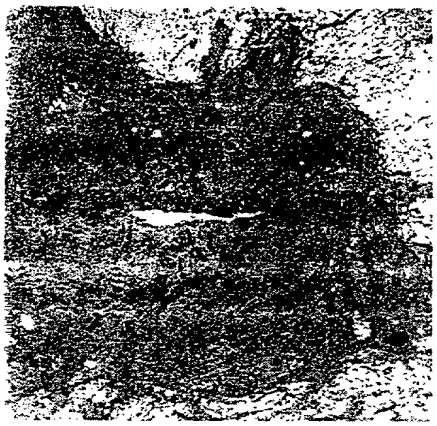


FIG. 5

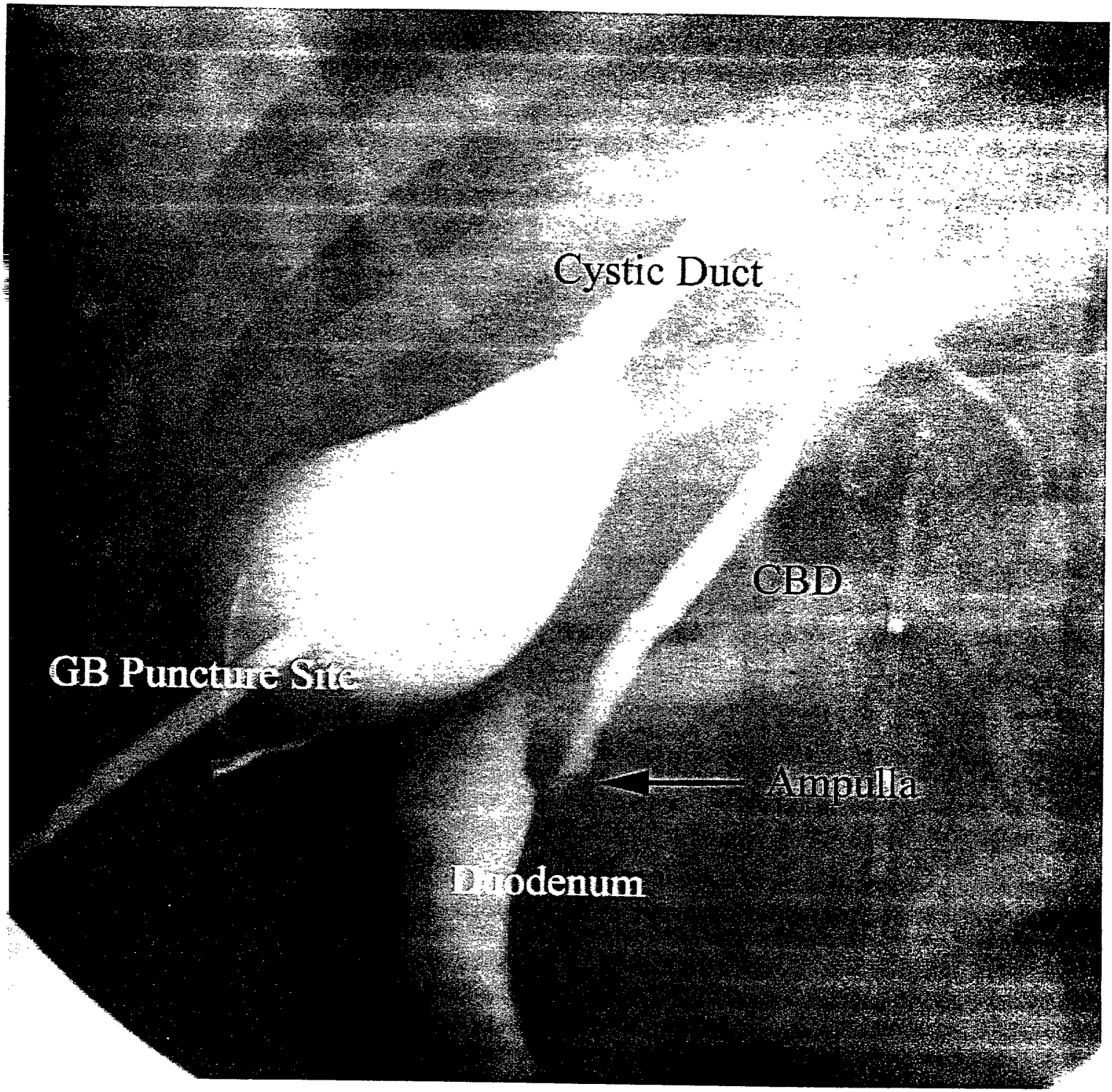


FIG. 6

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